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The Acute Toxicity of Thiocyanate and Cyanate to Rainbow Trout

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The Acute Toxicity of Thiocyanate and Cyanate to Rainbow Trout

Northern Affairs Program

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KEY WORDS

Project N-8303
Ecology
Effluent Treatment
Cyanide
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Cyanate
Acute Toxicity

EXECUTIVE SUMMARY

Thiocyanate and cyanate are both found in the final effluent of gold mill cyanide destruction plants, yet very little information exists about the possible toxic effects of these compounds on aquatic organisms.

On behalf of the Department of Indian Affairs and Northern Affairs Canada, and Noranda Inc., a series of bioassays with rainbow trout were conducted for the purpose of:

- determining the toxicity of thiocyanate and cyanate to rainbow trout under various conditions of temperature and water pH;
- determining if stress enhanced the toxicity of the two compounds;
- investigating the possible modifying effects of water hardness on the toxicity of thiocyanate and cyanate;
- investigating the toxicity of mixed thiocyanate/cyanate solutions.

The results of the study indicate that thiocyanate toxicity is affected by both temperature and water pH, the latter having the greater influence. To illustrate the range of results, the mean 96-h LC_{50} at pH 8 and 12°C was 263.2 mg/L SCN as compared to the mean LC_{50} of 178.4 mg/L SCN at pH 6 and 12°C.

Cyanate was substantially more toxic than thiocyanate in these tests, and its toxicity was also affected more by pH than temperature. The mean LC_{50} values ranged from 15.2 mg/L CNO at pH 6 and 5°C, to 80.6 mg/L CNO at pH 8 and 12°C. These data indicated that cyanate was two to twenty times as toxic as

thiocyanate depending on the pH and temperature of the test. The increased toxicity of cyanate at the lower pH most likely is associated with the production of ammonia from the breakdown of cyanate at pH 6. Stress at the end of the 96-h bioassay period increased the toxic response of trout to thiocyanate (but not to cyanate).

The results of the preliminary bioassays to examine the possible modifying effects of water hardness on the toxicity of thiocyanate and cyanate, suggest that increased water hardness reduces toxicity. The study was too limited to establish LC_{50} levels.

TECHNICAL SUMMARY

Thiocyanate and cyanate are both found in the final effluent of gold mill cyanide destruction plants, yet very little information exists about the possible toxic effects of these compounds on aquatic organisms.

On behalf of the Department of Indian and Northern Affairs Canada, and Noranda Inc., a series of bioassays with rainbow trout were conducted for the purpose of:

- determining the toxicity of thiocyanate and cyanate to rainbow trout under various conditions of temperature and water pH;
- determining if stress enhanced the toxicity of the two compounds;
- investigating the possible modifying effects of water hardness on the toxicity of thiocyanate and cyanate;
- preliminary investigation of the toxicity of mixed thiocyanate/cyanate solutions.

A series of 96-h static bioassays were conducted with rainbow trout (Salmo gairdneri) to assess the toxicity of thiocyanate and cyanate under several conditions of temperature and water pH. These tests were run at a water hardness of 75 mg/L (as $CaCO_3$) by proportionately mixing well water with deionized water. Another series of static bioassays were conducted to determine the effect of water hardness on the toxicity of thiocyanate and cyanate at constant conditions of temperature (12^{o}C) and water pH (8.0). Finally, a single test

was run to examine the possibility of synergistic and/or antagonistic action between the two toxicants.

The LC_{50} concentrations for bioassays conducted with SCN and CNO and their respective confidence intervals are given in the table below.

Toxicant	pH	Temp. °C	LC_{50} mg/L	95% Confidence Interval[1.] mg/L
SCN	8.0	5.0	223.5	204.4 - 242.6
SCN	8.0	5.0	210.8	198.6 - 223.0
SCN	8.0	12.0	259.0	241.2 - 276.8
SCN	8.0	12.0	259.4	247.6 - 271.2
SCN	8.0	12.0	271.2	258.2 - 284.1
SCN	6.0	5.0	225.8	216.3 - 235.3
SCN	6.0	5.0	277.4	264.3 - 290.4
SCN	6.0	12.0	184.3	176.8 - 191.8
SCN	6.0	12.0	172.4	165.7 - 179.0
CNO	8.0	5.0	45.7	39.7 - 51.7
CNO	8.0	5.0	43.4	38.2 - 48.6
CNO	8.0	12.0	78.2	70.4 - 86.1
CNO	8.0	12.0	105.9	91.2 - 120.6
CNO	8.0	12.0	62.5	52.4 - 72.6
CNO	6.0	5.0	18.5	15.5 - 21.5
CNO	6.0	5.0	7.3	6.0 - 8.7
CNO	6.0	12.0	42.1	34.9 - 49.3
CNO	6.0	12.0	24.0	21.1 - 26.9

1. Significant differences ($P < 0.05$ or less) existed among the results of each of the test conditions for either SCN or CNO.

The results of the tests run to establish the effect of temperature and water pH on the toxicity of thiocyanate are as follows:

- at pH 6, an increase in temperature (from 5 to 12°C) significantly increased the SCN toxicity (ie. lowered the mean LC_{50} from 251.6 to 178.4 mg/L SCN ($P<0.001$);
- at pH 8, an increase in temperature (from 5 to 12°C)

significantly reduced the SCN toxicity (ie. raised the mean LC_{50} from 217.2 to 263.2 mg/L SCN) ($P<0.001$);

- at a temperature of 5°C, an increase in pH (from 6 to 8) significantly increased the SCN toxicity (ie. lowered the mean LC_{50} from 251.6 to 217.2 mg/L SCN) ($P<0.05$);
- at a temperature of 12°C, an increase in pH (from 6 to 8) significantly decreased the SCN toxicity (ie. raised the mean LC_{50} from 178.4 to 263.2 mg/L SCN) ($P<0.001$).

The results of the tests run to establish the effect of temperature and water pH on the toxicity of cyanate are as follows:

- at pH 6, an increase in temperature (from 5 to 12°C) significantly reduced the CNO toxicity (ie. raised the mean LC_{50} from 12.9 to 33.1 mg/L CNO) ($P<0.025$);
- at pH 8, an increase in temperature (from 5 to 12°C) significantly reduced the CNO toxicity (ie. raised the mean LC_{50} from 44.5 to 82.2 mg/L CNO) ($P<0.005$);
- at a temperature of 12°C, and increase in pH (from pH 6 to 8) significantly reduced the CNO toxicity (ie. raised the LC_{50} from 33.1 to 82.2 mg/L CNO) ($P<0.001$);
- at a temperature of 5°C, an increase in pH (from pH 6 to 8) significantly reduced the CNO toxicity (ie. raised the LC_{50} from 12.9 to 44.5 mg/L CNO ($P<0.001$);

The increased toxicity of cyanate at pH 6 most probably resulted from the partial breakdown of CNO to ammonia.

Stress at the end of the 96-h bioassay period increased

the response of trout to thiocyanate but not to cyanate. In some cases, the response after stress was significantly greater, however, no correlation between increased response and specific test conditions was established.

The results of the preliminary bioassays to examine the possible modifying effects of water hardness on the toxicity of thiocyanate and cyanate, suggest that increased water hardness reduces toxicity. The study was too limited to establish LC_{50} levels.

The results of the bioassays at one set of conditions (pH 8, 12^{o}C, 75 mg hardness) with mixed thiocyanate/cyanate solutions indicate a possible enhancement of cyanate toxicity in the presence of thiocyanate. However, a general conclusion cannot be drawn from these results.

INTRODUCTION

Cyanidization has been used for extracting precious metals in many parts of the world for over a hundred years. Ores encountered in nature exhibit a wide variation of characteristics requiring many different processing approaches. In some instances, flotation must be employed, while in others, the ore may be leached directly with a dissolution agent (usually cyanide) at a high pH.

The cyanide concentrations are generally quite low (<1%), and the pH ranges between 10 and 12. In addition, the oxygen content of the leaching solution is maintained at a high level to aid the necessary oxidation of the precious metals (Ag, Au). In this highly oxidative environment, some of the cyanide is transformed to cyanate and thiocyanate.

Several methods are presently available for the treatment of cyanide-bearing effluents. The alkaline chlorination method does remove thiocyanate if sufficient chlorine is added to the barren solution. However, the SO_2 method recently proposed by Inco([1,2]) does not remove the dissolved thiocyanate. Therefore, any cyanide-bearing effluent, treated with SO_2, that is released to the aquatic environment, can contain thiocyanate.

Whereas there has been a significant amount of research reported in the literature on the toxic effects of cyanide on aquatic organisms, little information exists on the toxic effects of cyanates and thiocyanates in the aquatic environment. Hence, there is no valid basis for assessing the environmental merit of

alternative treatment methods in terms of aquatic toxicity.

The majority of the research has been summarized by Doudoroff (1976)([3]) as follows:

a) Brun (1936) - using KSCN found that concentrations greater than 59 mg/L SCN had harmful effects on Gambusia holbrooki (mosquito fish) in tests of 10-day duration.
- 2320 mg/L SCN killed the fish in 2 h.

b) Schaut (1939) - using KSCN found that 102 mg/L SCN killed unidentified minnows within 9 or 10 days.

c) Herbert (1962) - no rainbow trout died at 1800 mg/L SCN, however, the duration of the static bioassay was not given.

d) Demyanenko (1931) - 200 mg/L NH_4 SCN (153 mg/L SCN) killed bleak in about 50 h.
- 100 mg/L NH_4 SCN was not lethal to bleak in 144 h.

e) Shelford (1917) - 280-300 mg/L NH_4 SCN killed Lepomis humilis in 1 h.

f) Oshima (1931) - 780 mg/L NH_4 SCN (580 mg/L SCN) was tolerated by young eels (Anguilla japonica) for more than 25 h.
- a concentration 10 times as great killed the eels after 3.7 h of exposure.

g) Thumann (1950) - 100 mg/L NH_4 SCN (76 mg/L SCN) had no

effect on two rainbow trout after 10 h.

h) Walen et al (1957) - 96-h TLM of NH_4SCN to mosquito fish (*Gambusia affinis*) was 114 mg/L (87 mg/L SCN).

Doudoroff[3] concluded from his review of the literature on the toxicity of thiocyanate, that thiocyanate is a slow acting poison to fish. He also concluded that the toxicity of both the potassium and ammonia salts was the result of the thiocyanate ion in solution. More recently, bioassays with *Daphnia magna* indicated a 48-h LC_{50} of 51.4 mg/L SCN[4], whereas Anderson (1948)[5] reported that *Daphnia magna* were immobilized by concentrations of <11.3 mg/L as NaSCN. This suggests that *Daphna magna* may be much more sensitive to SCN than fish.

Even less information is available regarding the aquatic toxicity of cyanates. Values of 48.5 and 121 mg/L CNO were reported to be the tolerance level and the lower lethal limit for creek chub (*S. atromaculatus*) and unspecified fish respectively by Doudoroff[3]. However, test conditions under which these results were obtained were not included in publications that Doudoroff reviewed.

More recently, the Noranda Research Centre[6] undertook a preliminary study to examine the toxicity of cyanide, cyanate and thiocyanate to rainbow trout. The preliminary test results indicated 96-h LC_{50}'s for cyanide, cyanate and thiocyanate to be 0.066, 228 and 380 mg/L, respectively, expressed as the ion (that is, CN, CNO, SCN). The SCN results were confirmed by rainbow

trout bioassay conducted by EPS Halifax([7]); however, the results of their tests varied ± 50%. Subsequent to the initial submission of the proposal for this study, EPS conducted some additional relevant bioassays. No effect on SCN toxicity by pH of the testwater was noted, but the results did suggest a possible increased toxicity due to an upward shift in water hardness([8]). No tests were conducted to assess the effect of temperature on the toxicity of SCN to rainbow trout.

Stress-related enhancement of SCN toxicity had not been demonstrated by Noranda Research Centre studies([9]). However, work conducted by T. Heming, at the Co-operative Fishery Unit at Bozeman, Montana, suggests that stress in the way of stimulus to swim or instantaneous changes in lighting could evoke mortality at normally sublethal concentrations. Stress related 96-h LC_{50} values of 40-140 mg/L SCN have been recorded. Also, at concentrations as low as 10 mg/L SCN, blood plasma SCN concentrations were observed to increase to 140 mg/L. No living fish were observed to have blood plasma concentrations greater than 450 mg/L SCN([10]).

The present study, funded jointly by Dept. of Indian Affairs and Northern Development and Noranda Inc., examined the effect of temperature and pH on the toxicity of cyanate and thiocyanate to rainbow trout (Salmo gairdneri) in a series of static bioassays. Trends were also established delineating the modifying effect of water hardness on the toxicity of cyanate and thiocyanate. Fish stocks were held at either 6 or 13°C prior to

being used in bioassays at 5 and 12°C.

2. METHODOLOGY

2.1 Treated Well Water Characteristics

A constant supply of 11-12°C well-water supplied the bioassay laboratory. This water was passed through a green sand filter for the removal of excess iron; treated water typically had <0.1 mg/L total Fe. Other water quality characteristics are shown in Table I. Water in the bioassay tanks had approximately 0.25 the concentrations shown in Table I since it was filtered water blended with deionized water at an approximate rate of 3 parts deionized to 1 part filtered.

TABLE I - TREATED WELL WATER CHARACTERISTICS[1].

alkalinity as $CaCO_3$	275
hardness as $CaCO_3$	320
Cl	49.0
Na	161
Ca	66.1
Mg	35.1
K	12.0
Cu	<0.01
Zn	<0.01
Fe	<0.05
Total Dissolved Solids	889

[1]. expressed as mg/L

The bioassay laboratory was kept on a 12-h light-dark schedule with a combination of florescent and incandescent lights. An automatic dimmer was used to simulate dawn/dusk cycles to reduce the potential stress on the test fish resulting from the lights going on and off instantaneously.

2.2 Bioassay Methodology

Triplicated 96-h static bioassays (control and five

concentrations) on rainbow trout (Salmo gairdneri) were conducted in high density polyethylene tanks fitted into constant temperature baths. Test solutions were made up to 80 L by blending well water with deionized well water to achieve the desired test water hardness of 75 mg/L as $CaCO_3$. The pH of the test dilution water was adjusted in a large mixing vat and maintained at the desired level in the test vessels by the addition of 1 + 1 H_2SO_4 or 10 M NaOH. Aeration was supplied at a rate of 10 to 20 mL/min air.

The pH, dissolved oxygen and temperature were checked daily, while hardness, conductivity and test concentrations were recorded at the start and finish of each test. The pH was read using a Corning 120 pH meter; dissolved oxygen and temperature were read using a YSI Model 51B meter; hardness determinations were conducted with a Hach kit (confirmed to be within 10% of actual hardness by EDTA titration method([11])), while conductivity was measured using a YSI Model 33 meter.

The pH in the test vessels varied ± 0.1 units at pH 8 and ± 0.2 units at pH 6. Oxygen was observed to be between 80 and 100% saturation throughout the entire bioassay period. Hardness was kept at 75 mg/L as $CaCO_3$. Conductivity of control water was 250 umhos/cm, while for thiocyanate tests the conductivity ranged from 500 to 1000 umhos/cm, depending on toxicant concentration. In CNO tests, conductivity ranged from 310 to 500 umhos/cm. Water temperature, at the nominal temperatures of 5 and 12°C, varied ± 1°C throughout most tests.

Test solutions of KCNO and KSCN were made up from reagent grade chemicals by dissolving weighed-out portions into 500 mL - 1.0 L volumes of the dilution water and mixing these concentrated solutions with the water in the bioassay tanks. Test concentrations were measured either by a specific ion electrode method([11]) or a colourimetric procedure([12]) for CNO and SCN respectively (see Appendix B).

The mean weight of individual fish varied from 0.4 to 1.3 g during the entire experiment. Each replicate bioassay was conducted with 10 fish. Fish loading density throughout the experiment varied from 6.2 to 20 L/g/day with a mean of 14 L/g/day for an average fish weight of 0.57 g. Fish were placed into the bioassay tanks 48-h before the start of the test. Distribution was random. Fish were not fed 48-h prior to starting the bioassay, nor were they fed during the tests.

2.3 Hardness Bioassays

To establish whether a modifying effect of hardness on the toxicity of CNO and SCN, existed, several bioassays were conducted on each compound. Tests were conducted at the nominal pH value 8 and at 12^{o}C. Water hardness was adjusted to six different levels by blending various ratios of deionized and filtered mixtures. Each bioassay was conducted in duplicate plus a control was run at each hardness level. Ten fish per replicate were tested. The test hardnesses and the concentrations of either CNO or SCN are given in Table II.

TABLE II - WATER HARDNESS AND TOXICANT CONCENTRATIONS

Experiment Date	Water Hardness as mg/L $CaCO_3$ 1	2	3	4	5	6	Toxicant	(concentration) mg/L
25/06	25	75	150	200	275	350	SCN	(250)
15/10	25	50	75	150	250	355	SCN	(275)
1/10	25	75	100	200	275	355	CNO	(85)
8/10	25	50	75	100	125	150	CNO	(85)

2.4 CNO/SCN Mixtures

A single replicated bioassay was conducted at pH8, 12°C and 75 mg/L hardness was conducted to assess the potency of various mixtures of CNO/SCN. Proportions of the LC_{50} concentrations for CNO (75 mg/L) and SCN (275 mg/L) were blended together and tested in static bioassays for 96 h. The proportions corresponded to 0, 25, 50, 75 and 100% of the LC_{50} of each toxicant. That is 25% of the CNO LC_{50} concentration was added to 75% of the SCN LC_{50} concentration. The 100% proportions of the LC_{50} concentrations were used as controls to compare to previously determined LC_{50} values.

2.5 Stress

Bioassay fish were stressed subsequent to terminating the 96-h exposure period and making the final mortality observations. Stress was applied to the fish by chasing the surviving fish with a dipnet for 15 seconds, scooping them out of the tank, replacing them back into the tank. Fifteen minutes later, any additional mortalities were noted.

3. RESULTS AND DISCUSSION

3.1 Thiocyanate Bioassays

The results of bioassays conducted with rainbow trout fingerlings on KSCN are expressed as 96-h LC_{50} values in Tables III and IV. The 96-h LC_{50}'s for the pooled triplicate bioassays with 95% confidence intervals and the slope of the probit line are given in Table III. The 96-h LC_{50}'s for individual bioassays conducted at the two temperature levels and the two different water pH's are given in Table IV.

The results of the bioassays carried out under similar temperature and water pH conditions were compared to each other and to the results of bioassays conducted under the three other possible combinations of pH and temperature. These Comparisons were carried out using a one-way analysis of variance (ANOVA). The ANOVA tables are given in Appendix D. In addition to the above comparisons, all of the calculated LC_{50} values (from Table IV) were tested using an ANOVA and differences between means were determined using a Student-Newman-Keuls test (see Appendix C). The results of these comparisons indicated the following:

- at pH 6, an increase in temperature (from 5 to 12°C) significantly increased the SCN toxicity (ie. lowered the mean LC_{50} from 251.6 to 178.4 mg/L SCN) ($P<0.001$);
- at pH 8, an increase in temperature (from 5 to 12°C) significantly reduced the SCN toxicity (ie. raised the mean LC_{50} from 217.2 to 263.2 mg/L SCN) ($P<0.001$);
- at a temperature of 5°C, an increase in pH (from 6

TABLE III - 96-h LC_{50} Values and 95% Confidence Intervals for Pooled Replicate Bioassays with SCN (N = 30 fish/pool)

Experiment Date	pH[1]	Temperature[1] °C	Average Fish Weight g	96-h LC_{50} (S.E.)[2] mg/L	95% Confidence Interval mg/L	Slope
28/03	8.0	5.0	-	223.5 (9.77)	204.4 - 242.6	7.63
2/04	8.0	5.0	-	210.8 (6.23)	198.6 - 223.0	8.50
4/06	8.0	12.0	0.49	259.0 (9.10)	241.2 - 276.8	8.18
				217.4 (7.73)*	202.2 - 232.5	7.20
11/06	8.0	12.0	0.73	259.4 (6.00)	247.6 - 271.2	15.23
				248.1 (5.80)	236.7 - 259.5	15.39
13/07	8.0	12.0	0.54	271.2 (6.61)	258.2 - 284.1	10.94
				253.4 (6.50)*	240.7 - 266.1	10.03
16/04	6.0	5.0	-	225.8 (4.86	216.3 - 235.3	12.1
				158.4 (6.21)*	146.2 - 170.6	7.8
23/04	6.0	5.0	-	277.4 (6.67)	264.3 - 290.4	13.2
				234.4 (7.76)*	219.2 - 249.6	7.5
7/05	6.0	12.0	0.36	184.3 (3.82)	176.8 - 191.8	18.2
				182.2 (3.87)	174.6 - 189.8	18.0
21/05	6.0	12.0	0.48	172.4 (3.38)	165.7 - 179.0	20.9
				165.1 (3.86)*	157.5 - 172.6	15.8

[1] Nominal Values - Actual Values are Presented in Appendix B.
[2] Underlined Values are Those After Stressing the Test Fish.
* Indicates Statistical Difference from Unstressed Bioassay Results ($P<0.05$).

TABLE IV - LC_{50} Values for Individual Replicates

(N=10 fish/replicate) for SCN

pH	6.0		8.0	
Temp (°C)	Date of Experiment	LC50 mg/L	Date of Experiment	LC50 mg/L
5.0	16/04	221.6	28/03	209.3
		207.8		227.7
		247.2		237.0
	23/04	285.1	2/04	196.9
		259.0		220.8
		289.0		221.1
12.0	21/05	163.6	13/07	262.2
		174.0		270.6
		172.4		281.9
	7/05	195.6	4/06	241.0
		180.6		261.9
		178.9		283.8
			11/06	264.4
				267.6
				246.1

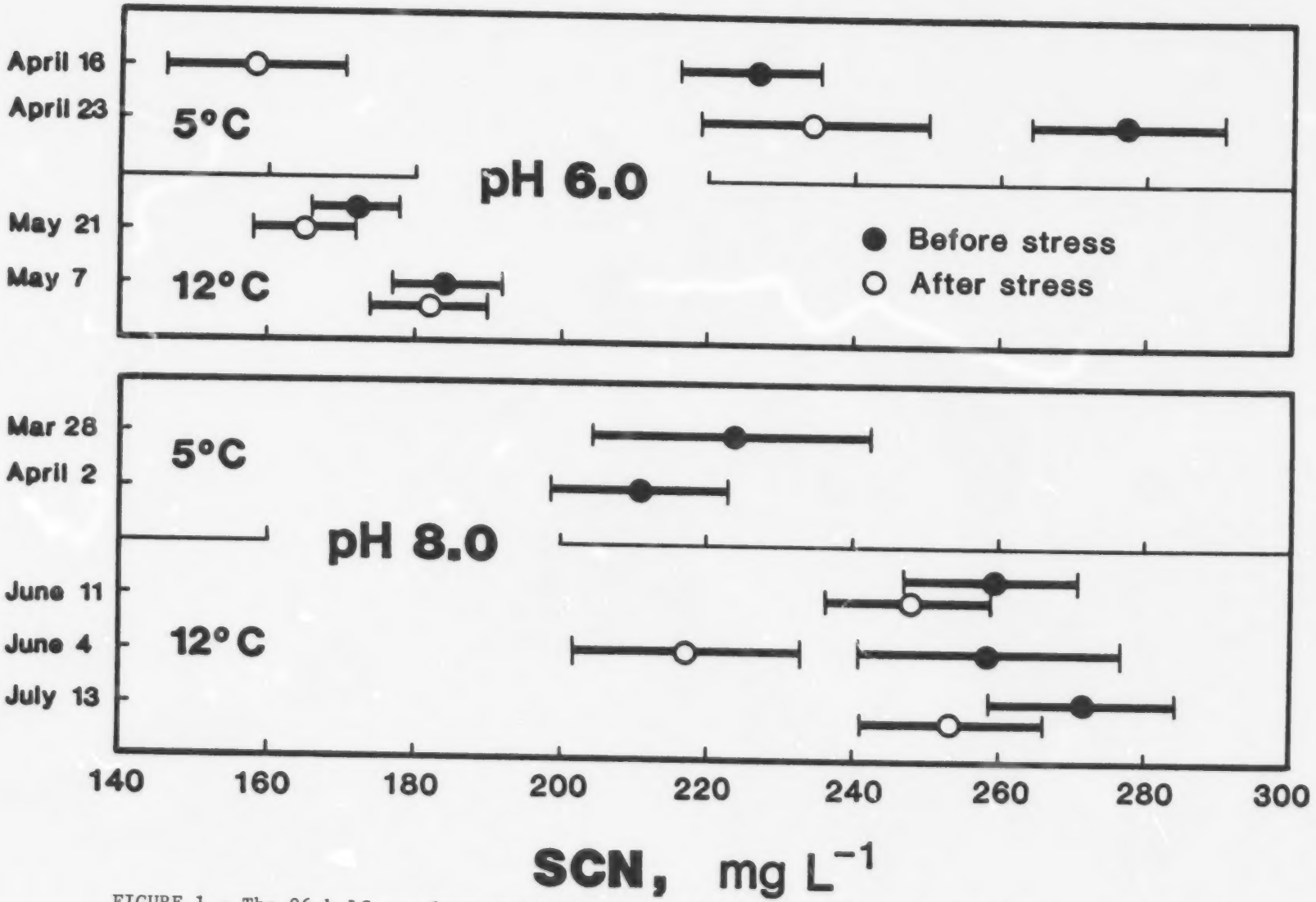

FIGURE 1 - The 96-h LC_{50} values and their respective 95% confidence limits for SCN bioassays (N=30)

to 8) significantly increased the SCN toxicity (ie. lowered the mean LC_{50} from 251.6 to 217.2 mg/L SCN) ($P<0.005$);

- at a temperature of 12°C, an increase in pH (from 6 to 8) significantly decreased the SCN toxicity (ie. raised the mean LC_{50} from 178.4 to 263.2 mg/L SCN) ($P<0.001$).

The results of the bioassays conducted at the two pH and two temperature conditions suggest SCN was most toxic when the pH was low (pH 6) and the temperature high (12°C). Since the pH of the solution would not affect the stability of the SCN, it is assumed that at this particular combination of test condition, changes in gill membrane permeability and metabolic rate are the most probable reasons for increased response to SCN. SCN was least toxic at the higher temperature (12°C) and at the higher pH level (pH 8). The data suggest that the pH of the test solution had a greater effect on the toxicity of SCN to rainbow trout than a change of temperature.

In tests conducted by the Environmental Protection Service (EPS) Halifax([8]), a similar response to different test water pH values was observed (see Table V). At a reduced water pH, SCN was observed to be more toxic to rainbow trout fingerlings during 96-h static bioassays.

TABLE V

A COMPARISON OF 1983 EPS DATA WITH THOSE OF THE PRESENT STUDY

FPS[8]				Present Study			
Water Hardness (mg/L as $CaCO_3$)	pH	Temp. °C	SCN-LC_{50} mg/L	Water Hardness mg/L	pH	Temp. °C.	SCN-LC_{50} mg/L
20	5.0	15	143	75	6.0	12	178
20	8.5	15	203	75	8.0	12	264

3.2 Effect of Stress on Thiocyanate Toxicity

Additional mortalities after the 96-h test period, due to stress, were observed at all of the test conditions (see Table IV). However, for tests conducted at pH 8 and at 5°C, additional mortalities were not documented because the effects were only observed after test fish had been transferred to a holding facility. There, an unrecorded proportion of the exposed fish died.

The LC_{50}'s for stressed fish were calculated using the number of mortalities observed during the 96-h bioassay plus those observed after stressing. These results are shown in Table III including statistical significance when compared to the results of unstressed fish. The LC_{50} values and their respective 95% confidence limits are illustrated and compared to results of unstressed fish in Figure I. In 5 out of 7 cases, the LC_{50}'s including stress were seen to be significantly ($P<0.05$) lower than those reported for SCN bioassays without stress.

There did not appear to be any pattern indicating a greater response to stress under any particular pH or temperature

condition. The results of EPS tests([8]) suggested a greater susceptibility to stress-induced toxicity at a higher pH, however, the data were too limited to confirm this trend.

The results of the present study and those of the study conducted by EPS indicated that SCN, with or without stress, was much less toxic than that previously reported by Heming et al([10]). Heming reported that concentrations as low as 8.0 mg/L SCN caused mortalities among rainbow trout exposed for 96-h and then stressed. Heming suggested that death of the stressed fish might be attributed to respiratory collapse during convulsions and a stress-induced shift in the thiocyanate-cyanide chemical equilibrium, resulting in the production of free cyanide in-vivo.

3.3 Cyanate Toxicity

The results of 96-h static bioassays conducted with rainbow trout fingerlings on KCNO are presented in Tables VI and VII and are shown in Figure 2. The 96-h LC_{50}'s for the pooled triplicate bioassays with their respective 95% confidence intervals and the slope of the probit line are given in Table VI. The 96-h LC_{50}'s for individual bioassays conducted at the two temperature levels and the two different water pH's are given in Table VII.

The results of the bioassays conducted at similar temperature and water pH conditions were compared to each other and to the results of bioassays carried out at the three other possible combinations of pH and temperature. The results were evaluated using ANOVA as was done for SCN. The ANOVA tables are

TABLE VI - 96-h LC_{50} Values and 95% Confidence Intervals for Pooled Replicate Bioassays with CNO (N=30 fish/pool)

Experiment Date	pH[1]	Temperature[1] °c	Average Fish Weight g	96-h LC_{50} (S.E.) mg/L	95% Confidence Interval mg/L	Slope
16/07	8.0	12.0	1.28	78.2 (3.98)	70.4 - 86.1	6.35
23/07	8.0	12.0	0.36	105.9 (7.51)	91.2 - 120.6	4.21
13/08	8.0	12.0	0.51	62.5 (5.16)	52.4 - 72.6	2.95
18/03	8.0	5.0	-	45.7 (3.04)	39.7 - 51.7	4.14
6/02	8.0	5.0	-	43.4 (2.64)	38.2 - 48.6	4.56
30/07	6.0	12.0	0.42	42.1 (3.69)	34.9 - 49.3	3.23
6/08	6.0	12.0	0.51	24.0 (1.50)	21.1 - 26.9	4.51
27/02	6.0	5.0	-	18.5 (1.55)	15.5 - 21.5	3.15
12/03	6.0	5.0	-	7.3 (0.69)	6.0 - 8.7	2.69

[1] Nominal Values - Actual Values are Presented in Appendix B.

TABLE VII - LC_{50} Values for Individual Replicates

(n=10 fish/replicate) for CNO

pH	6.0		8.0	
Temp (°C)	Date of Experiment	LC_{50} mg/kg	Date of Experiment	LC_{50} mg/kg
5.0	27/02	14.8	6/02	40.8
		21.2		46.4
		32.1		43.9
	12/03	7.4	19/03	34.9
		6.7		50.4
		7.9		55.7
12.0	6/08	21.3	16/07	78.2
		24.3		82.9
		27.7		74.6
	30/07	39.6	13/08	52.5
		33.5		52.0
		57.6		73.5
			23/07	96.2
				119.6
				95.5

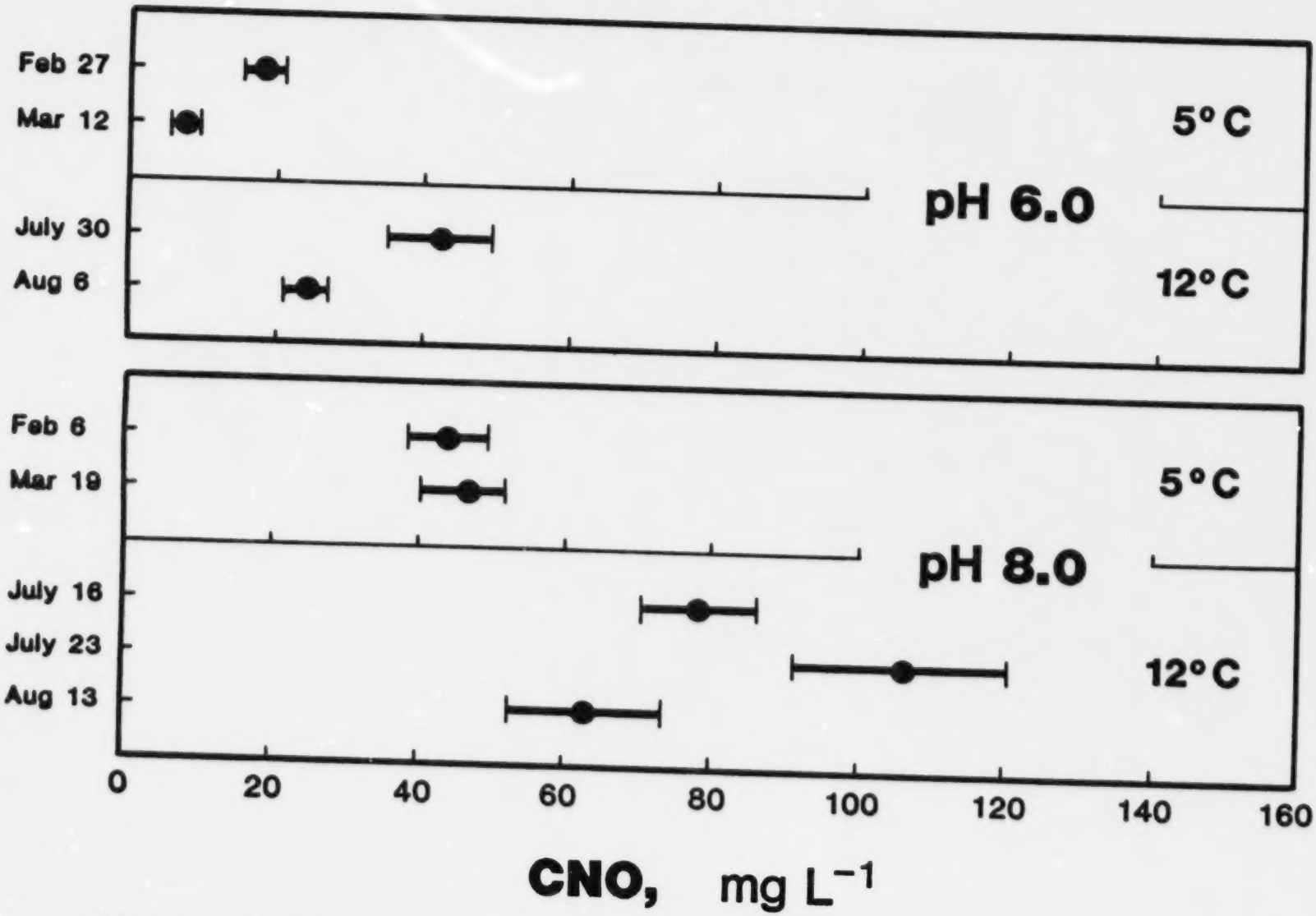

FIGURE 2 - The 96-h LC_{50} values and their respective 95% confidence limits for CNO bioassays (N=30)

provided in Appendix D. As well, a comparison of all LC_{50} values was done using an ANOVA, and mean LC_{50} values for each condition of pH and temperature were ranked and compared using a Student-Newman-Keuls test (Appendix C). The results of these comparisons indicated the following:

- at pH 6, an increase in temperature (from 5 to 12°C) significantly reduced the CNO toxicity (ie. raised the mean LC_{50} from 12.9 to 33.1 mg/L CNO) ($P<0.025$);
- at pH 8, an increase in temperature (from 5 to 12°C) significantly reduced the CNO toxicity (ie. raised the mean LC_{50} from 44.5 to 82.2 mg/L CNO) ($P<0.005$);
- at a temperature of 12°C an increase in pH (from pH 6 to 8) significantly reduced the CNO toxicity (ie. raised the LC_{50} from 33.1 to 82.2 mg/L CNO) ($P<0.001$);
- at a temperature of 5°C, an increase in pH (from pH 6 to 8) significantly reduced the CNO toxicity (ie. raised the LC_{50} from 12.9 to 44.5 mg/L CNO ($P<0.001$).

The above results show that the test condition under which CNO was most toxic to rainbow trout was when the water pH was 6 and temperature was 5°C. The next most toxic test condition occurred at pH 6 and 12°C. The least toxicity associated with CNO was observed at pH 8 and at 12°C.

It is apparent that pH had a greater influence on the outcome of the bioassays than did temperature. It is believed that during the lower pH studies, a small proportion of the CNO was converted to free ammonia which resulted in increased

toxicity of the test solutions. Cyanate can be hydrolyzed to carbonate and ammonia, especially at lower pH conditions([13]). The free ammonia was detected by specific ion electrode in samples of test water prior to treatment for CNO determinations. The basic mechanism on which the CNO determinations are based is the transformation of the nitrogen associated with the CNO to ammonia by acidifying and heating the samples. However, before treating the samples, baseline ammonia levels are established for each CNO determination. At pH 8, these baseline ammonia levels were always below the detection limit of the method (<0.1 mg/L), while ammonia levels in samples collected from tests conducted at pH 6 ranged between 0.5 and 1.5 mg/L. Un-ionized ammonia, at these concentrations has been demonstrated to be toxic to rainbow trout([14]).

It therefore appears that at the pH 6 level, fish had to contend with both CNO and ammonia toxicity and that possibly the ammonia exerted a greater toxic effect. Since baseline ammonia, levels at pH 8, were below the detection limit (<0.1 mg/L) it is assumed that mortalities were the result of exposure to CNO only.

Upon completion of the bioassays, fish were stressed as they were in the SCN tests, however, no observable effects of stress were recorded.

3.4 Effect of Water Hardness

The effects of water hardness on the toxicity of SCN and CNO are shown in Figures 3 and 4 respectively. In both

cases, the results of several tests for each compound, indicated that toxicity was reduced by increased water hardness.

This trend is the opposite of that reported by EPS([8]) for SCN, however, water quality parameters may be the main reason for the differences. EPS had to add $Ca(OH)_2$ to test water to achieve the desired hardness level. This addition resulted in an increase in pH which was adjusted by the addition of H_2SO_4. In the present study, hard well water (350 mg/L) was diluted with de-ionized well water to achieve the required hardness levels. No additions of $Ca(OH)_2$ or H_2SO_4 were required to achieve a particular water hardness level. Only a small addition of 10 M NaOH was made to adjust the dilution water pH from 7.5 to pH 8 for testing purposes.

The reduction in toxicity of SCN by water hardness was expected based on the work of Heming et al([10]) where it was suggested that there was direct competition in the transportation of chloride ions and SCN ions across the gill membranes. The chloride ion concentration during the hardness bioassays ranged between <0.1 mg/L (de-ionized water) to 49.0 mg/L (well water at 350 mg/L hardness). The relationship between mortalities and water hardness for tests conducted with 275 mg/L SCN (at $12^{o}C$, pH 8) is best described by a power curve where mortality $(y)=aX^{(b)}$ and where X=hardness.

The relationship between water hardness and toxicity of CNO is best described by the exponential formula $Y=ae^{bx}$, where Y=mortality and X=hardness. As was the case for SCN, there was a

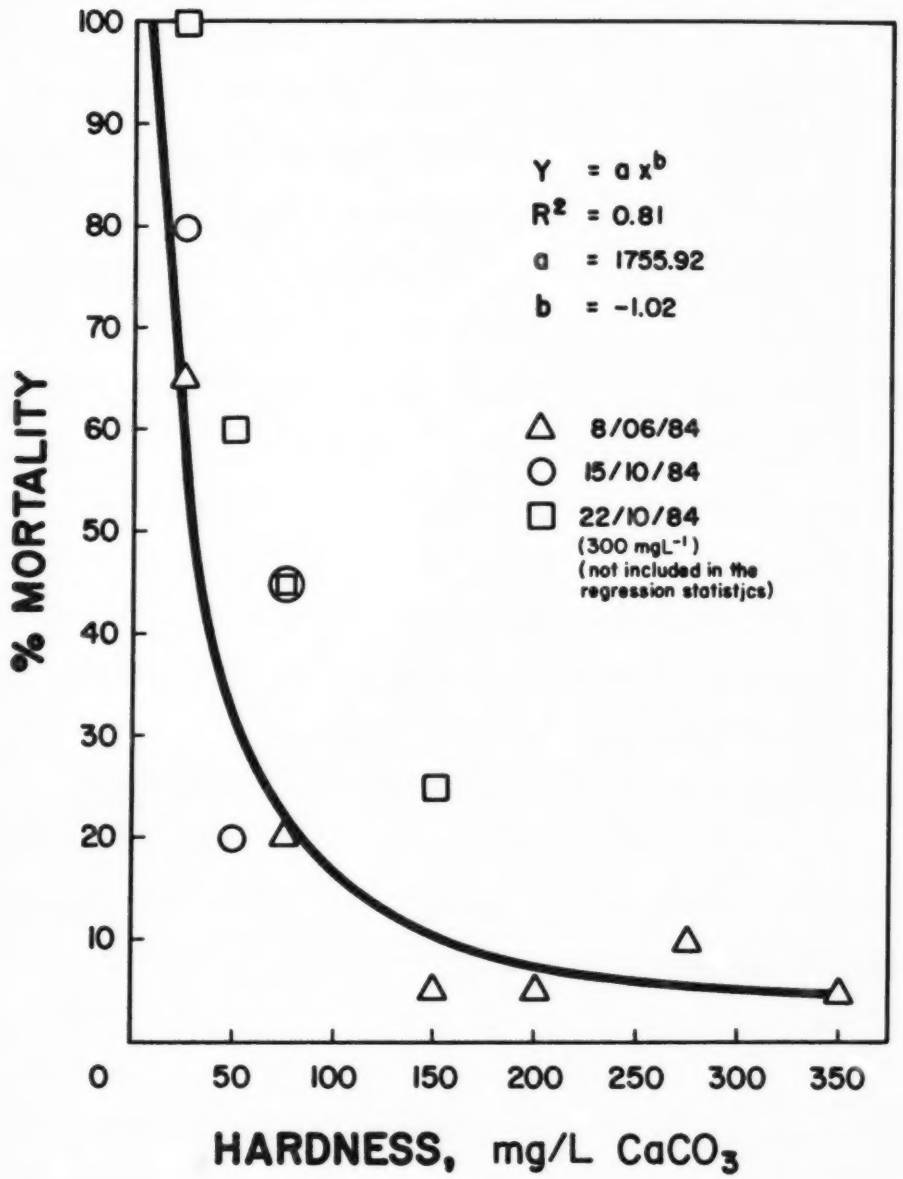

FIGURE 3 - A plot of % mortality against water hardness after 96-h exposure to 275 mg/L SCN.

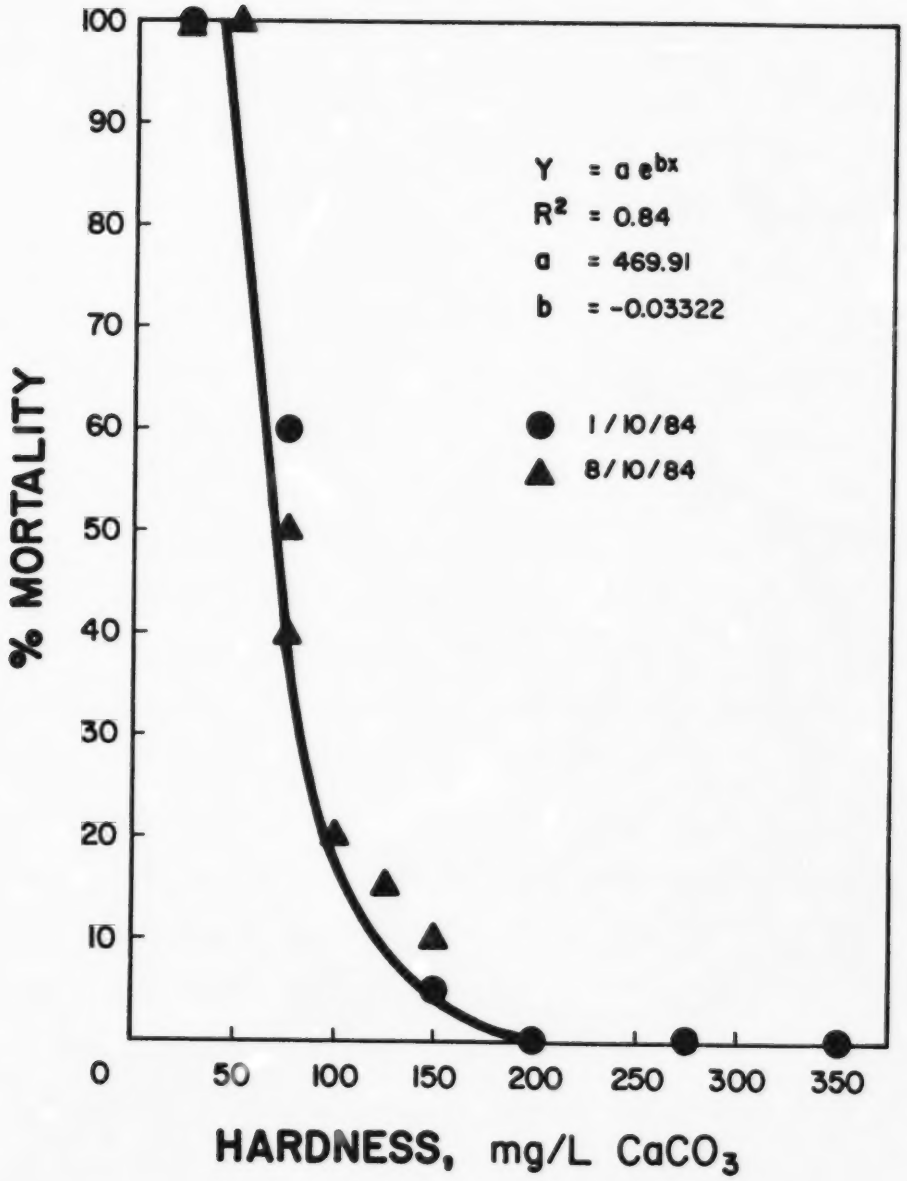

FIGURE 4 - A plot of % mortality against water hardness after 96-h exposure to 85 mg/L CNO.

reduction in toxicity of 85 mg/L CNO which was attributable to increased water hardness.

3.5 Thiocyanate/Cyanate Mixtures

The results of the bioassays conducted on mixtures of SCN and CNO are given in the table below.

Parts of CNO	Parts of SCN	% Mortality	% Mortality After Stress
4 (100%)	0 (0%)	45	45
3 (75%)	1 (25%)	44	44
2 (50%)	2 (50%)	41	56
1 (25%)	3 (75%)	48	52
0 (0%)	4 (100%)	0	43
0	0	0	0

The results of the bioassays (at one set of conditions, pH 8, 12^{o}C, hardness 75 mg/L) with mixed thiocyanate/cyanate solutions indicate a possible enhancement of cyanate toxicity in the presence of thiocyanate. The toxicity of the mixtures was as great as the toxicity observed for CNO by itself and greater than that observed for SCN. Stress at the end of the test increased the toxicity of SCN where it was present at a proportion greater than 25%. Further testing would be required to delineate more clearly the roles of SCN and CNO in toxicity tests conducted on mixtures of the two compounds.

4. CONCLUSIONS

The results of bioassays conducted with rainbow trout on SCN indicated that the pH and temperature of the test solutions could significantly affect the outcome of the assay. The most toxic of the test conditions was when the water pH was low (pH 6) and the water temperature high (12^{o}C). This suggests that the toxicity of the SCN in solution may have been enhanced by changes in gill permeability and metabolic activity attributable to these specific test conditions.

The results of bioassays run on CNO indicate that pH of the test solutions greatly influenced the toxicity to rainbow trout. CNO was observed to be more toxic at pH 6 than at pH 8, and elicited greater response at 5^{o}C than at 12^{o}C.

The results of the preliminary bioassays to examine the possible modifying effects of water hardness on the toxicity of thiocyanate and cyanate, suggest that increased water hardness reduces toxicity. The study was too limited to establish LC_{50} levels.

The results of the bioassays at one set of conditions (pH 8, 12^{o}C, 75 mg hardness) with thiocyanate/cyanate solutions indicate a possible enhancement of cyanate toxicity in the presence of thiocyanate. However, a general conclusion cannot be drawn from these results.

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APPENDIX A

Physico-Chemical Characteristics of the Bioassay Water

Physico-Chemical Characteristics of Bioassay Water (SCN)

EXPERIMENT DATE	PARAMETER [1]	MEAN [2]	SD
28/03	pH	7.9	0.2
	°C	4.6	0.4
	O_2	12.6	0.8
2/04	pH	8.2	0.4
	°C	4.8	0.4
	O_2	12.9	1.7
16/04	pH	6.1	0.2
	°C	6.5	0.8
	O_2	10.6	1.8
23/04	pH	6.1	0.1
	°C	6.8	0.6
	O_2	12.1	0.5
07/05	pH	6.1	0.2
	°C	11.7	0.4
	O_2	10.8	0.5
21/05	pH	6.1	0.1
	°C	11.6	0.3
	O_2	10.8	0.4
04/06	pH	8.0	0.1
	°C	12.2	0.3
	O_2	11.0	0.5
11/06	pH	8.0	0.1
	°C	12.3	0.3
	O_2	10.9	0.5
13/07	pH	8.0	0.1
	°C	12.0	0.3
	O_2	9.7	0.6

1. O_2 in mg L^{-1}

2. mean of all weekly readings for a particular parameter - $N \geq 72$.

Physico-Chemical Characteristics of Bioassay Water (CNO)

EXPERIMENT DATE	PARAMETER [1]	MEAN [2]	SD
6/02	pH	7.7	0.2
	°C	4.8	1.0
	O_2	-	-
20/02	pH	6.5	0.4
	°C	5.2	0.9
	O_2	-	-
12/03	pH	6.2	0.3
	°C	4.7	0.6
	O_2	-	-
19/03	pH	7.9	0.2
	°C	4.6	0.8
	O_2	-	-
16/07	pH	7.9	0.1
	°C	11.5	0.2
	O_2	10.5	0.6
23/07	pH	8.0	0.1
	°C	11.6	0.3
	O_2	10.6	0.6
30/07	pH	6.4	0.3
	°C	11.7	0.3
	O_2	11.2	0.3
6/08	pH	6.2	0.3
	°C	11.5	0.1
	O_2	10.8	0.3
13/08	pH	8.0	0.1
	°C	11.6	0.3
	O_2	10.3	0.4

1. O_2 in mg L^{-1}
2. Mean for all weekly readings of the particular parameter - N 372

APPENDIX B

Analyses of SCN and CNO Throughout the Bioassays

Percent Variation of Actual SCN Concentrations

From Nominal Levels

EXPERIMENT DATE		MEAN VARIATION %	S D %
23/04	Start	-3.6	3.1
	End	-2.5	2.1
4/06	Start	-5.3	2.4
	End	-3.6	2.5
25/06	Start	+1.1	2.2
	End	+3.4	2.4
9/07	Start	-0.6	3.1
	End	-1.3	2.7
11/06	Start	+3.2	2.0
	End	+0.1	2.3
07/05	Start	+0.8	1.4
	End	-2.2	2.2
21/05	Start	-1.9	2.5
	End	-3.6	3.8
02/04	Start	-16.0	10.7
	End	-0.2	3.8
28/03	Start	-3.2	12.3
	End	-15.6	6.4

Percent Variation of Actual CNO Concentrations

From Nominal Levels

EXPERIMENT DATE		MEAN VARIATION %	SD %
6/08	Start	+19.1	12.3
	End	-16.0	4.3
13/08	Start	-1.28	3.9
	End	-4.4	2.6
30/08	Start	-2.1	5.4
	End	+25.5	14.7
12/03	Start	+ 9.6	12.2
	End	+ 1.1	10.4
20/02	Start	+14.9	11.2
6/02	Start	+14.5	10.7
	End	+19.1	19.1

APPENDIX C

ANOVA Table and SNK Comparisons for SCN and CNO Bioassay Results

ANOVA Table and Student Newman-Keuls Comparison of Mean 96-h LC_{50} Values for CNS Toxicity Tests

Single Classification ANOVA with unequal sample sizes

Source of Variation	DF	SS	MS	F
Among Groups	3	30579.4	10193.1	27.24
Within Groups	23	8604.0	374.0	
Total	26	39183.5		

Significance of difference of means

		RANK	
R	Mean	N	4 1 3 2
4	264.38	9	
1	251.61	6	N
3	218.96	6	* *
2	177.51	6	* * *

* Tested and found to be significant
N Tested and found to be not significant

ANOVA Table and Student Newman - Keuls Comparison
of Mean 96-h LC_{50} Values for CNO Toxicity Tests

Single classification ANOVA with unequal sample sizes

Source of variation	DF	SS	MS	F
Among groups	3	17204.9	5734.9	24.73
Within groups	23	5332.8	231.8	
Total	26	22537.7		

Significance of difference of means

			RANK			
R	Mean	N	4	3	2	1
4	80.55	9				
3	45.35	6	*			
2	34.00	6	*	N		
1	15.21	6	*	*	*	

* Tested and found to be significant
N Tested and found to be not significant

APPENDIX D

Comparisons Between LC_{50} Values Using ANOVA

ANOVA TABLE FOR SCN

Comparison at pH6 of 96-h LC_{50} values at temperatures 5 ° and 12°C

ANALYSIS OF VARIANCE

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups	16472.43000	1	16472.43000
(F Value	27.52646)		
Within Groups	5984.21667	10	598.42167
Totals	22456.64667	11	

Level of Significance = 0.00038

Comparison at pH 8.0 of 96-h LC_{50} values at temperature 5° and 12 °C

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups	7427.44178	1	7427.44178
(F Value	36.85566)		
Within Groups	2619.86222	13	201.52786
Totals	10047.30400	14	

Level of Significance = 0.00004

ANOVA TABLE FOR SCN

Comparison at temperature 5°C of 96-h LC_{50} values at pH 6.0 and pH 8.0

ANALYSIS OF VARIANCE

Source of Variation	Sums of Square	D.F.	Mean Squares
Between Groups	3198.06750	1	3198.06750
(F Value	4.99027)		
Within Groups	6408.60167	10	640.86017
TOTALS	9606.66917	11	
Level of significance		0.04951	

Comparison at temperature 12°C of 96-h LC_{50} values at pH6.0 and pH 8.0

ANALYSIS OF VARIANCE

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups	27168.41878	1	27168.41878
(F Value	160.87138)		
Within Groups	2195.47722	13	168.88286
Totals	29363.89600	14	
Level of Significance		0.00000	

ANOVA TABLE FOR CNO

Comparison at pH 8.0 of LC_{50} values at temperature 5° and 12°C

Analysis of Variance

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups (F Value 14.65848)	4461.95211	1	4461.95211
Within Groups	3957.11722	13	304.39363
Totals	8419.06933	14	
Level of Significance		0.00209	

Comparison at pH 6.0 of LC_{50} values at temperature 5° and 12°C

Analysis of Variance

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups (F Value 7.76797)	1080.72120	1	1080.72120
Within Groups	1391.25300	10	139.12530
Totals	2471.97420	11	
Level of Significance		0.01921	

ANOVA TABLE FOR CNO

Comparison at temperature 5°C of 96-h LC_{50} values at pH 8.0 and 6.0

ANALYSIS OF VARIANCE

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups	2759.72670	1	2759.72670
(F Value	35.68544)		
Within Groups	773.34800	10	77.33480
Totals	3533.07470	11	
Level of Significance		0.00014	

Comparison at temperature 12°C of 96-h LC_{50} values at pH 8.0 and 6.0

Source of Variation	Sums of Squares	D.F.	Mean Squares
Between Groups	7802.71111	1	7802.71111
(F Value	22.17153		
Within Groups	4575.02222	13	351.92479
Totals	12377.73333	14	
Level of significance		0.00041	

APPENDIX E

Commentary by D.G. Dixon to the Soundness
of the Statistical Approach Used for Comparisons

University of Waterloo

Waterloo, Ontario, Canada
N2L 3G1

Faculty of Science
Department of Biology
519/885-1211

Telex Number
069-55259

22 November 1984

Mr. Menno R. Speyer,
Noranda Research Centre,
240 Hymus Blvd.,
Pointe Claire, Quebec
H9R 1G5

Dear Menno:

This letter constitutes the report with respect to the statistical analysis conducted on the results of bioassays with rainbow trout, on thiocyanate and cyanate, as requested in your letter of October 5, 1984.

The first step in assessing the material presented was to validate the calculation of LC50s from the raw data. This was completed for a random selection of the data provided. The analysis was undertaken using the SAS probit analysis package. The results are presented in Table 1; there were no meaningful discrepencies between the Noranda and Waterloo values.

Having established the validity of the LC50 calculations, the next step was to decide how to treat the data from experiments at the same pH and temperature which were carried out on different dates. There are two ways of dealing with this in analysis of variance. The first is to run a 3-way test (DATE, pH, TEMP.) and partition out that variability due to DATE. This has the disadvantage of seriously reducing N, leaving you with only 3 replicates in each cell. The second approach is to run a 2-way ANOVA (pH, TEMP) and allow the variability due to DATE to partition into the ERROR term. This approach was taken to maintain the higher number of replicates in each cell.

Two-way ANOVAs were carried out using the individual replicate data for SCN (your table III) and CNO (your table IV); tabulation of the results are enclosed. The results with CNO are identical to your own; the impacts of both temperature and pH were highly significant ($p<0.0001$). In addition, there was no significant interaction between temperature and pH. Therefore, it can be stated that both increased pH (6 to 8) and increased temperature (6 to 12) will result in significantly reduced CNO toxicity.

....2

The situation with the 2-way ANOVA for SCN was more complex. The pH influence was found to be significant, while the temperature effect was insignificant. There was, however, significant interaction between temperature and pH. I then carried out two 1-way ANOVAs (pH at 6°C; pH at 12°C) to check the source of the interaction (results enclosed). In both cases the impacts were significant. You are, therefore, safe in stating the conclusions regarding SCN which you outlined in your memo to me.

The final hypothesis to be tested involved the influence of "stress" on the 96-hr LC50s for SCN. There are several ways of evaluating this. The first is to run a 3-way ANOVA (pH, TEMP, STRESS). I was unable to do this since I did not have the individual LC50 values for each of the "stressed" replicates. The second method is the one which you applied (your Table 1), comparison of means using the "t" test. I checked three of the comparisons at random, and obtained results consistent with your own. A third method of comparison, and perhaps the best, is based on relative potency (see Finney, Probit Analysis). This involves pooling the raw data from each of the replicates of the bioassays to be compared. You might consider this for future work.

To summarize, the conclusions which you drew from the data would appear to be statistically defensible. If you require any clarification, feel free to call me.

Sincerely,

George

/kc

D. George Dixon, Ph.D.
Assistant Professor

Table 1. Comparison of Noranda and Waterloo LC50 calculations.

Bioassay	Replicate	Noranda		Waterloo	
		LC50 (mgL^{-1})	Fiducial limits (mgL^{-1})	LC50 (mgL^{-1})	Fiducial limits (mgL^{-1})
SCN 04/06	1	241.0	a	241.1	218.4-293.9
(pH, 8: 12°C)	2	261.9	a	261.9	b
	3	283.8	a	283.9	239.5-808.7
SCN 04/06	1	a	a	217.0	b
(pH, 8; 12°C	2	a	a	239.5	b
stressed)	3	a	a	253.2	217.1-495.4
SCN 13/07	1	262.2	a	262.2	243.2-289.5
(pH, 8; 12°C)	2	270.6	a	270.6	246.6-314.6
	3	281.9	a	282.0	b
SCN 13/07	1	a	a	232.3	211.7-249.4
(pH, 8; 12°C	2	a	a	263.6	236.0-310.6
stressed)	3	a	a	270.0	246.6-310.7

a. Noranda calculations not provided.

b. Data not suitable for calculation of fiducial limits.